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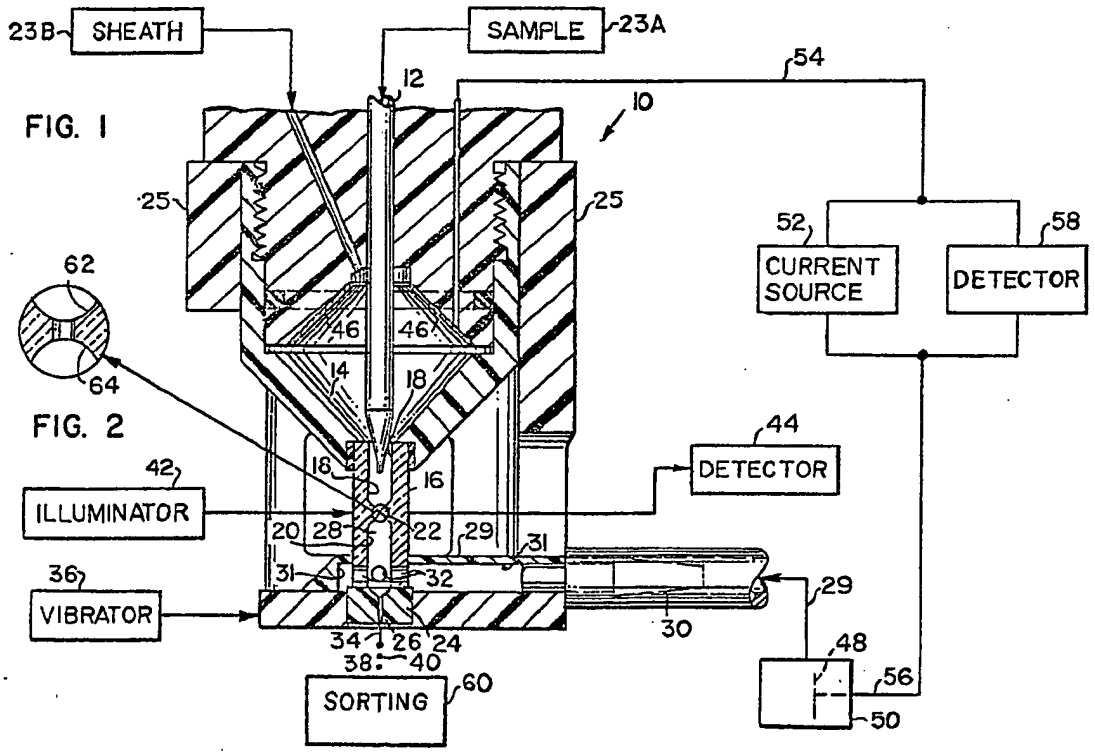
(54) **Particle analyzing and sorting apparatus**

(57) A particle analyzing apparatus for measurements of particles in a stream, comprising: a flow cell 16 having a pair of channels 18, 20, connected by an interposed particle sensing aperture 22 through which the particles pass, a nozzle 24 mounted proximate to the downstream end of the downstream one 20 of the pair of channels, and means 30, 31, 32 for introducing sheath liquid into the downstream end of the downstream channel 20 to flow into the nozzle exit orifice 26 and also towards the particle sensing aperture 22, so as to sheath and hydrodynamically focus the particle stream as it proceeds through the downstream channel 20 from the sensing aperture 22 to the nozzle 24.

FIG. 1



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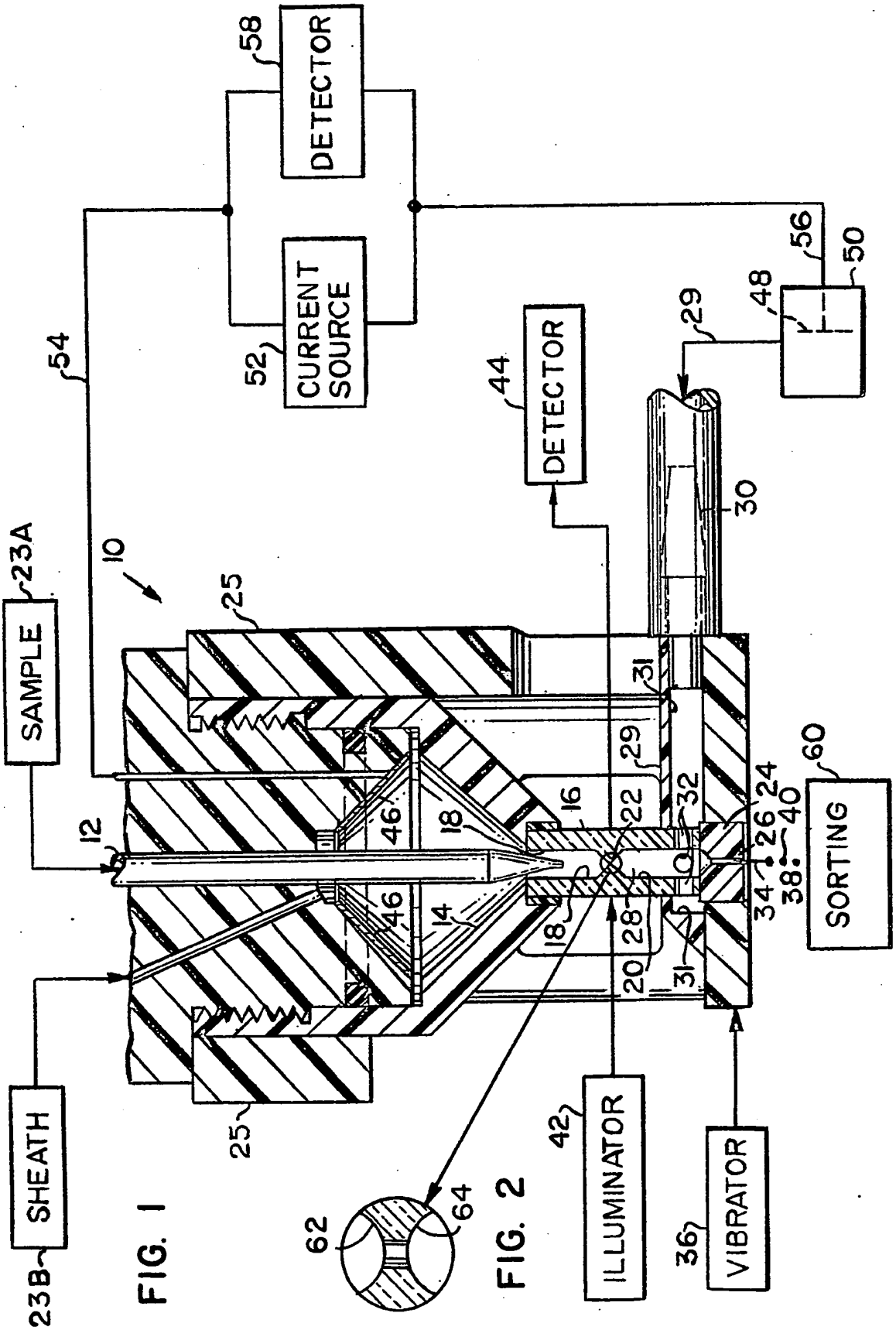
58
DETECTOR
52
CURRENT SOURCE
54
56
50
48
29
44
DETECTOR
30
23A
SAMPLE
10
25
31
29
60
SORTING
12
46
18
16
22
32
24
26
40
38
34
46
14
18
28
20
31
25
23B
SHEATH
42
ILLUMINATOR
36
VIBRATOR

FIG. 1

62
64

FIG. 2

SPECIFICATION

Particle analyzing & sorting apparatus

The invention relates generally to particle analyzing and sorting apparatus and more particularly is concerned with apparatuses in which studies may be made of particulate systems using the impedance sensing principle and optical measurements.

Since its conception in the early 1950's, the principle of particle counting and sizing invented by Wallace H. Coulter has resulted in numerous methods and flow-through apparatuses for the electronic counting, sizing and analysis of microscopic particles, which are scanned in a fluid suspension, as shown by the pioneer U.S. Patent 2, 656, 508 to Coulter. In this prior art arrangement, a D. C. electric current flow is established between two vessels by suspending electrodes in the respective bodies of the suspension fluid. The only fluid connection between the two bodies is through an orifice; hence, an electric current flow and field are established in the orifice. The orifice and the resultant electric field in and around it constitute a sensing zone. As each particle passes through the sensing zone, for the duration of the passage, the impedance of the contents of the sensing zone will change, thereby modulating the current flow and electric field in the sensing zone, and causing the generation of a signal to be applied to a detector suitably arranged to respond to such change.

For many applications of automated, flow-through particle analyzers, it is not possible to use just a small number of particle descriptors for identification of each type of cell present in a heterodisperse cell population of a sample. At present, many flow systems measure fluorescence, light scattering and electronic cell volume (impedance sensing). Additionally, there have evolved flow-through particle analyzers wherein the particles are positioned inside of liquid droplets and the droplets are sorted upon the above described measurements. Such sorting particle analyzers are shown in U.S. Patent 3,710,933 to Fulwyler *et al.* and in an article entitled "A Volume-Activated Cell Sorter", *The Journal of Histochemistry and Cytochemistry*, by E. Menke *et al.*, Vol. 25, pp. 796-803, 1977.

Major design problems are brought about by the use of both optical measurements and impedance measurements in the sorting particle analyzers. The above described sorting particle analyzers of the prior art perform electronic cell volume measurements prior to the optical measurements, making it necessary to correlate the two types of measurements. This correlation problem is not significant at very low particle flow rates; however, at high particle flow rates, it is possible for the detected signals to be scrambled by such artifacts as aggregates of cells which pull apart after they traverse a volume-sensing orifice, so as to move separately to the optical sensing zone, the presence of non-fluorescing particles; and the possibility of two neighboring cells exchanging position in the flow stream. Additionally, this requires the use of special circuitry for compensating for the time delay between the optical and electronic signals for a given particle.

Where sorting is not used, there has been developed a combined electro-optical particle analyzer in which all measurements are made simultaneously, thereby eliminating the complexity and uncertainty of correlating data obtained from sequential measurements. This electro-optical particle analyzer is described in an article entitled "Combined Optical and Electronic Analysis of Cells with AMAC Transducers", by Thomas et al., published in *The journal of Histochemistry and Cytochemistry*, Vol. 25, No. 7, (1977), pp 827-835. This multiparameter particle analyzer uses a square sensing orifice wherein all parameters are measured simultaneously. The square orifice is enclosed inside a cube formed by adhering four pyramids together.

The present invention provides:

A particle analyzing apparatus for studying particles in suspension, said apparatus including a flow cell having a particle sensing aperture through which a stream of particles in suspension is passed, said flow cell having an upstream channel and a downstream channel, with said particle sensing aperture being positioned therebetween and being the only fluid connection between said channels, exit means positioned proximate the downstream end of said downstream channel, and liquid introducing means constructed and positioned downstream of the downstream end of said downstream channel and operating for introducing liquid which flows both into said exit means as well as toward the upstream positioned particle sensing aperture for hydrodynamically focusing the stream of particles as it flows downstream from said particle sensing aperture into said exit means.

A preferred apparatus comprises a combined electro-optical particle analyzing and sorting apparatus, wherein both optical and electrical impedance (electronic volume) measurements are simultaneously made on a stream of particles passing through a particle sensing aperture. The flow cell has a pair of channels, an upstream channel and a downstream channel, defining openings at opposed ends thereof, with the particle sensing aperture fluidly connecting the two channels. The improvement in the apparatus comprises mounting a nozzle, containing an exit orifice, at the end of the downstream channel, so as to define a liquid-filled flow chamber. A sheath liquid is provided at the lower end of the flow chamber to provide a sheath for the particle stream over the entire length of the small diameter flow chamber, thereby leaving room in the flow cell, adjacent to the upper end of the downstream channel, for illuminating the particles and collecting light therefrom. By virtue of this design, the stream of particles are hydrodynamically focused as they proceed to the exit orifice and thereafter become part of a liquid jet. The liquid jet, in a conventional manner, is broken into a plurality of droplets, which are charged and sorted, based upon the above-described signals.

Heretofore, droplet sorting had never been included in an electro-optical apparatus wherein electrical impedance and optical measurements are

made simultaneously. Moreover, the applicants found that despite the small volume of the flow chamber, hydrodynamic focusing of the stream of particles by a liquid sheath could be accomplished by introducing the sheath liquid in the bottom of the downstream orifice and thereby not interfering with the optical assembly.

By way of example only illustrative embodiments of the invention now will be described with reference to the accompanying drawing in which:

Figure 1 illustrates a part cross-sectioned view and part block diagram of a particle analyzing and sorting apparatus according to the invention; and

Figure 2 is an enlarged cross-sectional view of the sensing aperture region of the flow cell of Figure 1.

Figure 1 illustrates a flow through, particle analyzing and sorting apparatus 10 having a sample introduction tube 12, a sheath tube 14 positioned in surrounding, coaxial relationship to the tube 12, and an optically transparent flow cell 16 positioned at the end of the tube 12. The flow cell 16 has formed therein a pair of opposed bores or channels 18 and 20 and a microscopic sensing aperture 22, which forms a fluid passageway between the ends of the channels. The aperture 22 defines a particle sensing zone to be described hereinafter. A liquid stream of individually suspended particles, originally from a pressurized reservoir 23A, proceeds through the tube 12. A saline laminar liquid sheath, originally from another pressurized reservoir 23B, proceeds through the tube 14 so as to surround the stream of particles. As the liquid stream of particles exits from the tube 12, and enters the first channel 18, hydrodynamic pressures reduce the diameter of the stream of particles as the stream obtains the velocity of the liquid sheath. The liquid sheath also acts to center the stream of particles so that particles pass through the orifice 22. After leaving the orifice 22, the particles enter the second channel 20, of the flow cell 16.

Various system components are supported by a cylindrical frame 25. A nozzle 24, with an exit orifice 26 formed therein, is mounted to the end of the flow cell 16, so that the nozzle 24 and second channel 20 define a liquid-filled flow chamber 28. A tube 29 is coupled to the frame 25 by a conduit fitting 30. A second sheath liquid is fed via the tube 29 to a liquid cavity 31, which is in fluid communication with three inlet orifices 32 formed in the wall of the flow cell 16. Due to the pressure drop associated with the aperture 22, it is necessary to introduce the second sheath liquid into the flow chamber 28 to create a second sheath having sufficient hydrodynamic pressures to pass the particles through the flow chamber 28 and out the exit orifice 26.

Contrary to the prior art designs, the second liquid sheath is introduced at the lower portion of the flow chamber 28, resulting in advantages in optical illumination and collection, which will be described hereinafter. More specifically, the sheath liquid enters the second channel 20 through the plurality of inlet orifices 32 positioned at locations a considerable distance below and downstream of the sensing aperture 22. Moreover, the second sheath liquid is introduced in a non-concentric manner relative to the particle stream exiting from the sensing aperture 22 and is injected into a relatively small interior volume of the second channel 20. Despite the small volume of the second channel 20 and the non-concentric introduction of the second liquid sheath at the bottom of the second channel 20, it has been found that a portion of the second sheath liquid travels "uphill" to capture the particle stream exiting from the sensing aperture 22, while a portion of the second sheath liquid goes immediately to the exit orifice 26 and all points in-between. In this manner, good hydrodynamic focusing of the particle stream through the flow chamber 28 is accomplished, therey allowing the stream to exit from the exit orifice 26. In this preferred embodiment, three inlet orifices 32 are shown. However, it should be understood that the number of orifices 32 are a mere matter of design choice, and one will suffice, although, depending upon their diameter, it is convenient to have more than one, so as to allow for cleaning and flushing of the flow chamber 28.

The system components shown schematically in block form are those which exist normally in conventional particle analyzer and sorting systems, sometimes referred to as flow cytometric sorting systems. Only those components of the particle analyzer and sorter 10 have been shown which are necessary to explain the operation of the present embodiment.

In a conventional manner, vibratory energy is applied to the liquid jet 34, exiting from the exit orifice 26, by vibratory means 36. As one possibility, the vibratory means 36 can comprise a piezo-electric crystal. The flow cell 16 is mounted to and supported by a piezo-electric crystal which vibrates the flow cell 16 at a high frequency. The exact frequency at which the cell 16 vibrates is dependent on the selected diameter of the exit orifice 26. These vibrations impart small disturbances, normally undulations, on the surface of the jet 34 which grow, due to well known surface tension effects, and eventually pinch the jet off at a breakoff point 38 into well defined droplets 40. The diameter of the exit orifice 26, the velocity of the liquid jet 34 and the dilution of the particle suspension are all predetermined so that normally there is no more than one cell in a given droplet 40.

By means of a conventional sorting arrangement 60, the selected droplets 40 are charged by, for example, a charging collar having a voltage applied thereto. Other droplets are not charged. The sorting arrangement 60 also includes a pair of deflector plates having an electrical potential difference applied therebetween. As the droplets pass between the plates, the charged droplets are deflected in the electric field, thereby allowing the charged droplets to be separated fom the uncharged droplets. The decision to charge a given droplet is based upon the previously described optical and impedance measurements for the particle contained within that droplet. The above description of the droplet forming and droplet sorting is only briefly given, since this portion of the apparatus 10 is well known in the art.

In the flow cell 16 the particle suspension is illuminated in a conventional manner, while passing

through the sensing aperture 22, by a light beam provided by an illumination source 42, for example a laser. The response of the particle in the sample suspension to the illumination, typically light scatter, fluorescence, or absorbance, is detected by an optical detector system 44. As is well known in the art, there are numerous illumination and light collection arrangements which can be used with the flow cell 16. However, by positioning the inlet orifice 32 substantially downstream of the aperture 22 according to the invention, the orifices 32 do not interfere with light illumination and collection; hence, greater solid angles of light illumination and collection are possible.

The sensing aperture 22 not only serves as an optical sensing zone as described above, but also serves as an electronic volume sensing zone, according to the principle of Wallace Coulter, as will be described below. An upstream electrode 46 is preferably mounted interiorly to the sheath tube 14. A downstream electrode 48 preferably is mounted in a remote chamber 50, which is in fluid communication with the flow chamber 28 through the tube 29. A low frequency current, including D.C., or high frequency current source 52 or both is electrically coupled to the electrodes 46 and 48 by way of electrical conductors 54 and 56 respectively. As the particles pass through the aperture 22, they modulate the electrical current so as to produce particle pulses detected by conventional detector circuitry 58. Illustrative current source 52 and detection circuitry 58 are shown in U.S. Patents 3,710,933; 3,502,974 and 3,502,973.

Preferably, but not necessarily, the channels 18 and 20 have a circular cross section of .05 inches with the overall length of the flow cell 16 being .25 inches. The flow cell 16 is formed from a monolithic piece of quartz, which allows for the flow cell 16 to be quite small. The smaller the size of the flow cell 16, the better its optical characteristics, in that the flow cell approaches a point source for the optical signals. The cross section of the particle sensing aperture 22 preferably aproximates a square. As seen in the further enlargement of Figure 2, the ends of the channels 18 and 20 are formed with spherical surfaces 62 and 64, which are each interrupted by the aperture 22. By providing rounded ends for the bores, the aperture 22 does not have to be precisely located. The outside surfaces are flat and parallel to the walls of the aperture 22. Typically, the aperture 22 has walls with lengths of 50 to 100 micrometers. Preferably, the aperture 22, the exit orifice 26 and the channels 18 and 20 are coaxially aligned. The above-described dimensions and configurations described in this paragraph are merely illustrative and can assume other shapes and sizes, respectively.

Although the apparatus is used primarily for studying cells, it is equally applicable to other kinds of particles.

Although particular embodiments of the invention have been shown and described herein, there is no intention thereby to limit the invention to the details of such embodiments. On the contrary, the intention is to cover all modifications, alternatives, embodiments, usages and equivalents of the subject invention as fall within the spirit and scope of the invention, specification and the appended claims.

CLAIMS

1. A particle analyzing apparatus for studying particles in suspension, said apparatus including a flow cell having a particle sensing aperture through which a stream of particles in suspension is passed, said flow cell having an upstream channel and a downstream channel, with said particle sensing aperture being positioned therebetween and being the only fluid connection between said channels, exit means positioned proximate the downstream end of said downstream channel, and liquid introducing means constructed and positioned downstream of the downstream end of said downstream channel and operating for introducing liquid which flows both into said exit means as well as toward the upstream positioned particle sensing aperture for hydrodynamically focusing the stream of particles as it flows downstream from said particle sensing aperture into said exit means.

2. A particle analyzing apparatus according to claim 1 in which said liquid introducing means is constructed and arranged with respect to the stream of particles so as to introduce the liquid in a non-concentric manner.

3. A particle analyzing apparatus according to claims 1 or 2 in which said liquid introducing means is constructed and arranged with respect to the stream of particles such that the liquid enters said flow cell at approximately right angles to the stream of particles.

4. A particle analyzing apparatus according to any one of claims 1, 2, or 3 and further including: illumination means for providing radiation to illuminate particles in said particle sensing aperture, said illumination means and its radiation being oriented and positioned substantially remote from said liquid introducing means.

5. A particle analyzing apparatus according to any one of claims 1 to 4 in which said liquid introducing means is positioned proximate to said exit means and said exit means includes a nozzle having an exit orifice for jetting the stream of particles as a liquid jet from said nozzle.

6. A particle analyzing apparatus according to claim 5 including disturbing means for periodically disturbing the liquid jet to produce droplets containing particles and sorting means for sorting said droplets, said sorting means being controlled by signals generated when the particles pass through said particle sensing aperture.

7. A particle analyzing apparatus for studying particles in suspension, the apparatus being substantially as herein described with reference to, and as illustrated by, the accompanying drawing.

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